Abstract

Introduction: In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods of analysis of immunosuppressive drugs have proven superior because of their increased sensitivity and selectivity. We evaluated the ability of an ultrafast SPE/MS/MS system to simultaneously analyze tacrolimus, sirolimus, and cyclosporin A in whole blood with much faster sample cycle times and similar analytical results compared to LC/MS/MS assays.

Methods: MS methods for tacrolimus, sirolimus, and cyclosporin A and their corresponding internal standards were optimized for analysis by QQQ MS. Calibration standards for each analyte were prepared in bovine whole blood. The whole blood samples were mixed with water and precipitated using a zinc sulfate and methanol solution containing the internal standards. Precipitated samples were gently mixed and then centrifuged. Following centrifugation, supernatants were transferred to a 96-well plate for analysis.

Results: Prepared calibration standards were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Cyclosporin A, for example, had both intra- and inter-day accuracies within 15% and coefficient of variation values less than 6% for all concentrations within the linear range (7.8-10.0 ng/ml). The method for all four analytes had excellent linearity within their respective measured ranges with an R² value greater than 0.995. Signal-to-noise ratios were calculated by looking at peak to peak height and found to be greater than 40:1 at the limit of quantification for all four analytes. To further evaluate this method, identical human samples were analyzed by RapidFire and a traditional LC/MS/MS method. Excellent correlation was found for the two methods.

Conclusions: Based on these results: tacrolimus, everolimus, sirolimus, and cyclosporin A can be accurately and precisely measured in whole blood. All four immunosuppressant drugs were simultaneously analyzed in a 12 MRM panel in less than 13 seconds per sample using ultrafast SPE/MS/MS. While the analytical results were comparable to LC/MS/MS, the analysis time was approximately 10 times faster. This methodology is capable of throughputs >270 samples per hour.

Introduction and Experimental

We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze tacrolimus, sirolimus, and cyclosporin A in whole blood. The SPE/MS/MS system consisted of an Agilent RapidFire 360 and an Agilent 6460 triple quadrupole mass spectrometer. The RapidFire cycle consisted of five states: aspirate, load/aqueous wash, extra wash with 50% methanol, elute, and re-equilibration (Table 1). All four immunosuppressant drugs and their corresponding internal standards were simultaneously measured using a 12 MRM panel (Table 2).

Table 3. QQQ MS Conditions

![Table 3. QQQ MS Conditions](image)

Experimental

Calibration standards for tacrolimus, sirolimus, everolimus (0.8-50 ng/mL), and cyclosporin A (15.6-1000 ng/mL) were prepared by spiking all four drugs into bovine whole blood. Commercially available quality control standards made in whole blood were prepared according to the manufacturer’s instructions. The whole blood samples were mixed with water and precipitated using a zinc sulfate and methanol solution containing the internal standards. Precipitated samples were gently mixed and then centrifuged. Following centrifugation, supernatants were transferred to a 96-well plate for analysis.

Results and Discussion

Figure 4. Representative standard curve and intra- and interday data for Cyclosporin A

![Figure 4. Representative standard curve and intra- and interday data for Cyclosporin A](image)

The RapidFire method for all four analytes had excellent linearity within their respective measured ranges with an R² value greater than 0.995. Signal-to-noise ratios were calculated by looking at peak to peak height and found to be greater than 40:1 at the limit of quantitation for all four analytes. To further evaluate this method, identical human samples were analyzed. The human samples were independently analyzed by LC/MS/MS at UCL and aliquots of identical samples analyzed by RapidFire. Excellent correlation was found for the two methods.

Figure 5. Correlation of human samples analyzed by LC/MS/MS vs. RapidFire SPE/MS/MS

![Figure 5. Correlation of human samples analyzed by LC/MS/MS vs. RapidFire SPE/MS/MS](image)

Conclusions

Cyclosporin A, everolimus, sirolimus, and tacrolimus were accurately and precisely measured in whole blood. All four immunosuppressant drugs were simultaneously analyzed in a 12 MRM panel in less than 13 seconds per sample. Human samples correlated well with identical samples ran independently by a traditional LC/MS/MS method. While the analytical results were comparable to LC/MS/MS, the analysis time was approximately 10 times faster. This methodology is capable of throughputs >270 samples per hour.